Evaluation of a biological marker of dairy fat intake

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ABSTRACT  We evaluated whether the adipose tissue content of 2 fatty acids of exogenous origin specific for ruminant fat, 15:0 and 17:0, reflect average long-term dairy fat consumption in free-living subjects. In 81 healthy women aged 30–77 y, we compared the relative content of these 2 fatty acids in subcutaneous adipose tissue with relative intake (% of total fat) based on four 1-wk weighed diet records made 3–4 mo apart and on a food-frequency questionnaire reflecting average past year consumption. The mean (±SD) daily milk fat intake was 20.0 ± 9.1 g and fat from ruminant meat was 3.0 ± 1.5 g according to food records, representing 29.2 ± 8.9% and 4.6 ± 2.2% of total fat, respectively. The intake of 15:0 and 17:0, which are 1.05% and 0.61% of milk fat and 0.43% and 0.83% of ruminant meat fat, was 0.22 ± 0.10 and 0.15 ± 0.06 g, respectively. Content of 15:0 and 17:0 in adipose tissue was 0.35% and 0.24% and relative dietary intake was 0.33% and 0.22% according to the food records and 0.32% and 0.21%, respectively, according to the food-frequency questionnaire. Correlation coefficients between 15:0 content in adipose tissue and intake from dairy foods only, according to food records, were 0.63 (Pearson) and 0.59 (Spearman); corresponding values for 17:0 were 0.42 and 0.45, respectively. Content of 15:0 and 17:0 in subcutaneous adipose tissue might be a valid biological marker of long-term milk fat intake in free-living individuals in populations with high consumption of dairy products.  Am J Clin Nutr 1998;68:291–5.

KEY WORDS  Milk fat, adipose tissue, biological marker, validity, dairy fat, weighed food records, food-frequency questionnaire, saturated fat, ruminant fat, pentadecanoic acid, heptadecanoic acid

INTRODUCTION

Interest in the health effects of dietary fat encompasses the amount and type of fat consumed by individuals and populations. Epidemiologic studies of dietary fat and disease, both observational and interventional, would benefit from biochemical indicators of fat consumption. Although no satisfactory biochemical measure of total fat consumption has been identified, the fatty acid composition of subcutaneous adipose tissue reflects the type of fats consumed by different populations in international comparisons (1), groups within countries (2, 3), and individuals (4–7). The highest correlations have been observed for polyunsaturated fatty acids, which are largely exogenous, whereas saturated fatty acids and monounsaturated fatty acids may be synthesized endogenously.

In the search for additional biomarkers, we decided to investigate the validity of 2 saturated fatty acids with odd numbers of carbon atoms, ie, pentadecanoic acid (15:0) and heptadecanoic acid (17:0), as markers for intake of dairy fat. These fatty acids are synthesized by the bacterial flora in the rumen of ruminants (8, 9). Because these fatty acids contain an uneven number of carbon atoms, they cannot be synthesized in the human body and are virtually specific for milk fat. Although they are present in low concentrations, it is possible to quantitate the proportions of 15:0 and 17:0 in human adipose tissue. Because these fatty acids are not produced endogenously, they may possess the characteristics required for a biological marker.

In the present study we evaluated whether the content of 15:0 and 17:0 in adipose tissue might reflect average long-term dairy fat consumption from milk and milk products in free-living subjects. We compared the fatty acid composition of adipose tissue (the relative content of these 2 fatty acids) with four 1-wk weighed diet records and with a food-frequency questionnaire (FFQ). The use of the 2 methods of dietary assessment of long-term consumption not only served as 2 measurements against which the fatty acid composition of adipose tissue could be compared, but it also provided an opportunity to compare the relative performance of the diet-assessment methods with that of an independent measure.

SUBJECTS AND METHODS

Subjects

The study was approved by the Ethical Committee at Uppsala University Hospital. The 741 female subjects, 30–77 y of age, were randomly selected within 5 age strata (30–39, 40–49, 50–59, 60–69, and 70–77 y) from the total population register of Uppsala county, central Sweden. The women were invited to...
participate in a validation study of an FFQ, in which four 1-wk weighed food records were used as a reference method. Because of the high degree of cooperation required and time-consuming procedures, only 213 women participated in the validation study. Of these participants, 196 completed all four 1-wk diet records between March 1991 and December 1992. At the conclusion of the fourth week of diet recording, a subcutaneous fat biopsy was obtained from the lateral buttck from the first 84 consecutive women who completed all four 1-wk diet records. For 81 women, all 3 measurements—the FFQ, diet records, and adipose tissue aspirate—were available and our analyses are based on these subjects. This subsample was comparable with the original random sample in age (mean of 55 y compared with 54 y, respectively).

Adipose tissue collection and fatty acid analyses

Subcutaneous fat aspirate samples were taken from the upper, outer quadrant of a buttck of each subject with a needle attached to a vacuum tube. The samples (≈10–30 mg) were stored frozen at −70°C, protected from light, and analyzed within a few weeks (10). The adipose tissue biopsies were weighed, dissolved in 1 mL hexane, and homogenized in a tissue grinder for 15 min. The hexane was pipetted off and the solvent was evaporated to dryness. The fatty acids were separated by gas-liquid chromatography after transmethylation as described earlier (11). The fatty acid methyl esters were separated on a 25-m wall-coated, open-tubular, glass capillary column coated with SLP OV-351, with helium as a carrier gas. A Hewlett-Packard system (Avondale, PA) consisting of model GLC 5890, integrator 3396, and autosampler 7671A was used.

The fatty acids were identified by comparing retention times with those of NuCheck Prep (Elysian, MN) fatty acid methyl ester standards and polyunsaturated fatty acid mix no. 2 (Supelco, Bellefonte, PA). In assays with adipose tissue fatty acids, CVs were 5.3% for 15:0 and 9.1% for 17:0; for comparison, the CV for 18:2 was 0.7%.

Dietary records

An experienced dietitian provided detailed instructions to small groups of participants or to individuals about weighing and recording all foods consumed. The dietary records were kept for four 1-wk periods 3–4 mo apart during the year after the administration of the semiquantitative FFQ. Each study participant was provided with an electronic scale, a set of plastic standard household measures of volume (1 dL, 0.5 dL, 15 mL (1 tablespoon), 5 mL (1 teaspoon), and 1 mL), and a food diary (including detailed written instructions). Participants were encouraged to use mainly the electronic scale. After the return of each 1-wk record, the dietitian reviewed the diary and telephoned the participants, if needed, to resolve ambiguities.

The dietary records were entered into a computer for analysis by using the personal computer nutrient software package MATS (12). For nutrient calculations we used the Swedish Food Administration food database, PC version 1992, which includes 1593 foods and dishes (13). For reported dishes not included in this database, the dietitian obtained recipes from the participants and entered appropriate amounts of the component foods. The database provides information on total fat and 14 specific fatty acids. However, this database does not include 15:0 and 17:0. We estimated the total milk fat intake directly by summarizing the amount of fat from all dairy products (milk, sour milk, yogurt, cheese, cream, and butter) and from dishes including these products (taking into account the amount of specific milk products in the recipe). Then we estimated the amounts of 15:0 and 17:0 specifically by using the assumption, based on empirical data, that they represent 1.05% and 0.61%, respectively, of total milk fat. These percentages are based on analyses of 73 milk samples obtained from 10 commercial dairy herds from the 2 different regions in Sweden. The samples were collected at afternoon milkings, immediately frozen at −20°C, and transported to the laboratory (P Barrefors, unpublished observations, 1997). Furthermore, we estimated the total fat intake from ruminant meat (beef and lamb). We estimated the amounts of 15:0 and 17:0 from this source by using the assumption that they represent 0.43% and 0.83%, respectively, of ruminant fat.

These percentages are based on analyses of 5 samples of ruminant meat obtained from different butchers and analyzed in the laboratory at the Department of Food Science, Swedish University of Agricultural Sciences (J Jiang, personal communication, 1997).

Food-frequency questionnaire

We used an 88-item FFQ with relative portions. For foods usually eaten on a daily basis—such as milk (5 types), bread (4 types), cheese (6 types), coffee, sugar, and fat on sandwiches—we asked open questions, ie, numbers of glasses of milk, slices of bread, slices of cheese, cups of coffee, and teaspoons of sugar per day or week. For fat on sandwiches we asked the participants whether they usually used a thick, thin, or very thin layer. For the other food items listed in the questionnaire, participants were asked to estimate the frequency of consumption and indicate what portion size they usually ate (small, medium, or large) in relation to a specified standard portion for each food item. The standard portions corresponded with “natural” units (eg, 1 orange, 2 eggs) or typical serving sizes derived from weight tables for foods and dishes prepared by the Swedish Food Administration (14).

For the frequency of food consumption there were 9 predefined frequency categories, ranging from “never or less than once per month” to “three or more times per day.” The questionnaire also included additional questions about type of fat on the table (5 types) and fat usually used in cooking (8 types). For nutrient calculations, missing frequency answers were treated as the “never or less than once per month” category. Daily energy and nutrient intakes were calculated by multiplying the frequency of consumption of each food by the indicated portion size and by the nutrient content of each food item (or a weighted average nutrient composition of each food group), and then summing across all foods. The total milk fat intake and intake of 15:0 and 17:0 were estimated directly in a way similar to that used for the food records.

Statistical analyses

The analyses were based on 81 subjects for whom all 3 measurements (adipose tissue aspirate, diet records, and FFQ) were available. The means ± SDs are presented for fat and specific fatty acids. To evaluate the association between intake of 15:0 and 17:0 in the diet and their relative content in adipose tissue, we used Pearson product-moment correlation coefficients. Natural logarithmic transformations were used to improve normality. Because not all data reached complete normality, Spearman correlations are presented as well. The SAS program was used for analyses (SAS Institute Inc, Cary, NC).
Ruminant fat 17:0 were similar to their proportions in milk fat, namely, 1.5, glasses (200 mL) of low-fat (0.5% fat) milk. be 4 portions of cheese, 4 small portions (5 g) of butter, and 2 of cheese (20 g = 2 thin slices) and for the highest intake it could records). Typical food eaten for the lowest intake was 1 portion presented by total milk fat, ruminant fat, and 15:0 and 17:0 were almost identical. records). Mean absolute estimates of milk fat, fat from ruminant meat, and 15:0 and 17:0 intake based on the FFQ were underreported in comparison with the diet records according to the FFQ was 44–46 g/d, respectively, corresponding to 41.9% of total fat intake. Mean saturated fat intake – 0.16% for 15:0 and 0.61 – 0.1% for 17:0. The content of these 2 fatty acids in ruminant fat also seems to be quite stable (SDs are not large): 0.43 – 0.09 and 0.83 – 0.14, respectively (J Jiang, personal communication, 1997).

In Table 2, the relations between intake of fatty acids and adipose tissue composition are presented. A higher Pearson correlation between adipose tissue composition and the intake of the corresponding fatty acids calculated from diet records and the FFQ was observed for 15:0 than for 17:0. Use of the sum of 15:0 and 17:0 intake based on the FFQ were underreported in comparison with the diet records (Table 1). However, the proportions of total fat intake represented by total milk fat, ruminant fat, and 15:0 and 17:0 were almost identical.

Total intake of milk fat varied from 6 to 46 g/d (based on food records). Typical food eaten for the lowest intake was 1 portion of cheese (20 g = 2 thin slices) and for the highest intake it could be 4 portions of cheese, 4 small portions (5 g) of butter, and 2 glasses (200 mL) of low-fat (0.5% fat) milk.

With both diet-assessment methods, ratios between 15:0 and 17:0 were similar to their proportions in milk fat, namely, 1.5, and were similar to the ratio observed in adipose tissue of = 1.5. Content of 15:0 and 17:0 in milk fat is quite stable, which is reflected by relatively low SDs of percentage estimates: 1.05 ± 0.16% for 15:0 and 0.61 ± 0.02% for 17:0. The content of these 2 fatty acids in ruminant fat also seems to be quite stable (SDs are not large): 0.43 ± 0.09 and 0.83 ± 0.14, respectively (J Jiang, personal communication, 1997).

In Table 2, the relations between intake of fatty acids and adipose tissue composition are presented. A higher Pearson correlation between adipose tissue composition and the intake of the corresponding fatty acids calculated from diet records and the FFQ was observed for 15:0 than for 17:0. Use of the sum of 15:0 and 17:0 as a biomarker did not increase the correlation coefficients. This means that 15:0 alone is as valid as the sum of 15:0 and 17:0. Direct correlations between daily total dairy fat intake based on food records and 15:0 in adipose tissue were 0.63 (Pearson; Figure 1) and 0.59 (Spearman), correlations with the sum of 15:0 and 17:0 in adipose tissue were 0.62 and 0.60,

**Table 1**

<table>
<thead>
<tr>
<th>Fat or fatty acid</th>
<th>Diet records</th>
<th>Food-frequency questionnaire</th>
<th>Adipose tissue</th>
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</thead>
<tbody>
<tr>
<td><strong>Milk fat</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(g/d)</td>
<td>20.0 ± 9.1 (5.9–46.0)</td>
<td>13.8 ± 10.5 (0.2–48.4)</td>
<td>–</td>
</tr>
<tr>
<td>(% of total fat)</td>
<td>29.4 ± 8.9 (12.8–53.4)</td>
<td>29.5 ± 14.2 (1.7–66.1)</td>
<td>–</td>
</tr>
<tr>
<td><strong>Ruminant fat</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(g/d)</td>
<td>3.0 ± 1.5 (0.2–7.6)</td>
<td>1.5 ± 1.0 (0.1–4.2)</td>
<td>–</td>
</tr>
<tr>
<td>(% of total fat)</td>
<td>4.6 ± 2.2 (0.6–11.5)</td>
<td>3.5 ± 2.0 (0.4–10.9)</td>
<td>–</td>
</tr>
<tr>
<td>15:0</td>
<td>0.22 ± 0.10 (0.08–0.50)</td>
<td>0.15 ± 0.11 (0.003–0.53)</td>
<td>–</td>
</tr>
<tr>
<td>(% of total fat)</td>
<td>0.33 ± 0.09 (0.17–0.57)</td>
<td>0.32 ± 0.15 (0.02–0.70)</td>
<td>0.35 ± 0.07 (0.21–0.59)</td>
</tr>
<tr>
<td>17:0</td>
<td>0.15 ± 0.06 (0.06–0.32)</td>
<td>0.10 ± 0.07 (0.003–0.33)</td>
<td>–</td>
</tr>
<tr>
<td>(% of total fat)</td>
<td>0.22 ± 0.05 (0.12–0.34)</td>
<td>0.21 ± 0.08 (0.02–0.41)</td>
<td>0.24 ± 0.05 (0.09–0.34)</td>
</tr>
<tr>
<td>15:0 + 17:0</td>
<td>0.37 ± 0.15 (0.14–0.82)</td>
<td>0.25 ± 0.18 (0.006–0.86)</td>
<td>–</td>
</tr>
<tr>
<td>(% of total fat)</td>
<td>0.55 ± 0.14 (0.30–0.90)</td>
<td>0.53 ± 0.23 (0.05–1.10)</td>
<td>0.58 ± 0.10 (0.38–0.91)</td>
</tr>
</tbody>
</table>

1 SD; range in parentheses. n = 81. In calculations, milk fat from milk, sour milk, yogurt, cheese, cream, and butter as well as from dishes with dairy products such as pizza, pancakes, and lasagna is taken into account; ruminant fat is from beef and lamb meat.

2 Pearson and Spearman correlation coefficients of relative fatty acids intake (% of total fat) calculated from four 1-wk weighed diet records and a food-frequency questionnaire, with fatty acid composition of subcutaneous adipose tissue in women.

3 n = 81. The milk group included milk, sour milk, yogurt, cheese, cream, and butter; the other dairy group included dishes with dairy products, eg, pizza, pancakes, and lasagna; the ruminant group included beef and lamb meat.

4 Based on log-transformed values.

5 P < 0.0001.

6 P < 0.05.

7 P < 0.001.
The individual data for daily milk fat intake (% of total fat) and 15:0 content in adipose tissue are presented in Figure 2.

DISCUSSION

Our results extend previous observations of a correlation between dietary intake of specific fatty acids with the composition of subcutaneous fat. The fatty acid composition of adipose tissue is considered a valid biomarker for the intake of polyunsaturated fatty acids (15), which might be superior to food records (16). The strength of the relations in our study between the estimated intakes of 15:0, 17:0, and their sum, and the corresponding fatty acid proportions in adipose tissue is of a similar order (16) or stronger (15) than those described earlier between the intake of polyunsaturated fatty acids and adipose tissue fatty acids. Hunter et al (7) reported a correlation coefficient of 0.49 between two 7-d diet records and fat aspirate measures of polyunsaturated fatty acids for men from the Boston area; Tjønneland et al (17) reported a correlation of 0.74 for Danish men. Studies in women reported a correlation of 0.37 when adipose tissue composition was correlated with a semiquantitative FFQ (15), 0.46 compared with two 7-d weighed food records (17), and 0.68 compared with 19 different 24-h recalls (5). In our study in women we found a correlation of 0.72 between polyunsaturated fatty acid content in adipose tissue and dietary intake estimated from four 7-d weighed food records and 0.67 for intake estimates based on an FFQ (16). We conclude that the adipose tissue content of the saturated fatty acid 15:0 or the sum of 15:0 and 17:0 is a valid biomarker for intake of these specific fatty acids as well as a good indicator of intake of total dairy fat. The high correlation observed for 15:0 as compared with dietary records (Pearson \( r = 0.63 \)) reflects the fact that this fatty acid is of exogenous origin; 17:0 alone has slightly less value as a biomarker (\( r = 0.42 \)).

To our knowledge, there are no published data on the relation between the dietary intakes of 15:0 and 17:0 and their adipose tissue content that reflect the validity of adipose tissue composition as a biological marker of the intake of these fatty acids. Finding a biological marker for consumption of milk fat may be of considerable importance for nutritional epidemiologic studies, both observational and interventional. Objective measurement of intake might be superior to estimates based on self-reported data. Milk fat is of special interest in the development of coronary artery disease because of its atherogenic and thrombogenic properties; 67% of milk fat consists of saturated fatty acids (18). In ecological studies, per capita consumption of milk and milk products is significantly correlated with increased coronary artery disease mortality rates (19). However, many prospective cohort studies do not confirm the expected positive association of saturated fat with this chronic disease (20). In fact, there are...
some studies suggesting a hypocholesterolemic effect of milk products (21–24) whereas others show that substitution of skim milk for whole milk may decrease the risk of coronary artery disease (25). These discrepancies may partly result from poor validity of dietary assessment of the consumption of dairy products based on subjective self reports. Therefore, finding a valid, objective biological marker of dairy fat intake might be of great value in clinical and epidemiologic nutrition studies.

In our study population, milk fat represented 29.4% and meat fat from ruminants 4.6% of the total fat intake. The small percentage from ruminant fat explains why adding information about intake of 15:0 and 17:0 from ruminant fat did not increase correlations. However, these relations between total dietary intake of 15:0 and 17:0 and adipose tissue composition might be different in populations with a high intake of ruminant fat (beef and lamb) and low intake of milk fat. In such populations, information about ruminant fat intake might influence correlations with adipose tissue composition markedly and content of 15:0 in adipose tissue might not be a good biological marker of milk fat intake specifically. However, we can expect that it would be a good marker of total 15:0 intake or even a marker of beef and lamb meat intake in populations consuming few or no dairy products.

REFERENCES